

Genetic and Environmental Factors Associated with Variation of Human Xenobiotic Glucuronidation and Sulfation

Brian Burchell and Michael W.H. Coughtrie

Department of Biochemical Medicine, Ninewells Medical School, The University, Dundee, Scotland

Glucuronidation and sulfation are phase 2 metabolic reactions catalyzed by large families of different isoenzymes in man. The textbook view that glucuronidation and sulfation lead to the production of harmless conjugates for simple excretion is not valid. Biologically active and toxic sulfates and glucuronides are produced and lead to adverse drug reactions, including immune hypersensitivity. Considerable variation in xenobiotic conjugation is observed as a result of altered expression of UDP-glucuronosyltransferases (UGTs) and sulfotransferases (STs). Recent cloning and expression of human cDNA encoding UGTs and STs has facilitated characterization of isoform substrate specificity, which has been further validated using specific antibodies and human tissue fractions. The availability of cloned/expressed human enzymes and specific antibodies has enabled the investigation of xenobiotic induction and metabolic disruption leading to adverse responses. Genetic polymorphisms of glucuronidation and sulfation are known to exist although the characterization and assessment of the importance of these variations are hampered by appropriate ethical studies in man with suitable safe model compounds. Genetic analysis has allowed molecular identification of defects in well-known hyperbilirubinemias. However, full characterization of the specific functional roles of human UGTs and STs requires rigorous kinetic and molecular analyses of the role of each enzyme *in vivo* through the use of specific antibodies and inhibitors. This will lead to the better prediction of variation of xenobiotic glucuronidation and sulfation in man. — *Environ Health Perspect* 105(Suppl 4):739–747 (1997)

Key words: polymorphisms, enzyme induction, inhibition, gene families, cloned/expressed enzymes, antibodies, toxic conjugates, acylglucuronidation

Introduction

Glucuronidation and sulfation of pharmacologically active xenobiotic compounds and endogenous substances is a major phase 2 detoxication system in man and has profound effects on the disposition, metabolism, and excretion of many drugs (1,2).

Glucuronidation is a major detoxication pathway in all vertebrates. Glucuronide formation is catalyzed by a family of UDP-glucuronosyltransferases (UGTs) using thousands of endobiotic and xenobiotic compounds as substrates (3). The xenobiotic substrate range of an individual UGT isoform may be dictated by the evolved structure of an individual UGT to accept

endobiotic substrates such as bilirubin or a steroid (4). A thorough understanding of the evolved endobiotic UGT substrate range is essential. Although an extensive list of drugs is glucuronidated by humans (3), it remains difficult to determine and predict UGT specificity. However, the fallibility of the evolved detoxication systems has been revealed by the implication of drug glucuronides in adverse drug reactions that resulted in hypersensitivity of immune response (5).

The cytosolic sulfotransferases (STs), derived from the ST gene superfamily, catalyze the sulfation of a host of xenobiotics

and endogenous compounds, including steroids, bile acids, thyroid hormones, and neurotransmitters (2). Sulfotransferases of various kinds may be found in species as diverse as bacteria and man. Although in mammals sulfation, in common with most phase 2 reactions, is generally considered to serve a detoxication function, it also plays a role in, for example, steroid biosynthesis (6). For certain compounds (e.g. aromatic hydroxylamines and hydroxamic acids, benzylic alcohols of polycyclic aromatic hydrocarbons) sulfation is the terminal step in their bioactivation to mutagens (7). Many xenobiotics are substrates for both UGTs and STs, and the different subcellular location and kinetic properties of the enzymes and the availability of cosubstrate influence the relative contribution of each system.

The availability of cloned human UGTs and STs has allowed for significant progress (3), although our excitement needs to be tempered by our lack of knowledge of the contribution of each isoform to xenobiotic conjugation *in vivo*. Moreover, the use of "rate" data obtained using recombinant cell lines may lead to prediction of *in vivo* pharmacokinetics, although there are a number of limitations that have to be circumvented (8).

This minireview will assess recent work on the interindividual variation of glucuronidation and sulfation in man caused by genetic differences in expression of UGTs or STs. Further, we shall briefly examine the bioactivation of xenobiotics following conjugation with sulfate and glucuronic acid as a mechanism of potential toxicity in man.

The UGT Family of Genes and Enzymes

More than 14 human liver UGT cDNAs have been cloned and classified into two subfamilies based on sequence analysis. The UGT1 subfamily of enzymes glucuronidate xenobiotic phenols and bilirubin, while UGT2 enzymes glucuronidate steroids and bile acids. An early observation was that UGT1 cDNA clones shared an identical C-terminal coding sequence whereas the N-terminal 246 amino acids show a striking lack of identity (24 vs 49%) (9).

Southern blot analysis indicated that the region encoding the conserved 3' half of four separate human UGT1 cDNAs (the common domain) was a single copy in the human genome, suggesting a role for alternative splicing in the synthesis of different

This paper was prepared as background for the Workshop on Susceptibility to Environmental Hazards convened by the Scientific Group on Methodologies for the Safety Evaluation of Chemicals (SGOMSEC) held 17–22 March 1996 in Espoo, Finland. Manuscript received at EHP 5 November 1996; accepted 18 November 1996.

We thank The Wellcome Trust (BB) and the Commission of the European Communities (Contracts BMH1-CT92-0097; EV5V-CT94-0410, MWHC) for their support of the research work in our laboratories.

Address correspondence to Dr. B. Burchell, University Department of Biochemical Medicine, Ninewells Hospital and Medical School, DUNDEE DD1 9SY, Scotland, UK. Telephone: 44 1382 632164. Fax: 44 1382 644620. E-mail: bburchell@ninewells.dundee.ac.uk

Abbreviations used: CN, Crigler-Najjar syndrome; GS, Gilbert's syndrome; PAPS, 3'-phosphoadenosine 5'-phosphosulfate; ST, sulfotransferase; UGT, UDP-glucuronosyltransferase; YAC, yeast-affiliated chromosome.

isoforms. In support of this, the common domain and the isoform-specific 5' half of the four UGT1 cDNAs colocalized to chromosome 2 at 2q.37 (10). Owens et al. (11) described the existence of a gene complex by the isolation of overlapping cosmid clones containing six alternative substrate-determining first exons upstream of the four exons that make up the common domain. The human liver cDNA clone HP4 isolated by Wooster et al. (12) had the same common domain sequence as other UGT1 cDNAs but contained a novel substrate-determining exon that was termed UGT1*02 and was not among those already genomically cloned. This suggested that the *UGT1* gene locus was larger than had previously been described, presumably extending further upstream. Human genomic Southern blotting indicated the presence of multiple sequences homologous to the 5' portion of *UGT1*02* (13). Further work has shown that the human *UGT1* gene is a single-copy gene that consists of 4 common exons and more than 13 variable exons that span more than 200 kb of the human genome (C Brierley and B Burchell, unpublished data).

In contrast, comparison of members of the *UGT2* gene family, the steroid metabolizing isoforms, indicates that amino acid differences between different isoforms of this family occur throughout the length of the protein. Three *UGT2B* genes were mapped to chromosome 4 using somatic cell hybrid cell lines and polymerase chain reaction. To determine whether they were clustered we screened a yeast-affiliated chromosome (YAC) library and isolated a five-YAC contig. One of these YACs was found to contain at least three *UGT2B* genes in 195 kb. Fluorescence *in situ* hybridization analysis indicated that the *UGT2B* gene cluster was localized at 4q13 (14).

Use of Cloned and Expressed Human Liver UGTs to Determine Substrate Specificity

Human UGT isoforms have been identified by gene sequencing and cDNA cloning (9), but only a few of these are known to be expressed *in vivo*. Indeed, one sequence is known to contain stop codons that would prevent functional enzyme expression (9). Consequently, only a few human UGTs have been substantially characterized.

Six cloned human hepatic UGT cDNAs were stably expressed in tissue culture cell

lines. More than 100 drug xenobiotics and endobiotics were used as substrates for glucuronidation catalyzed by the cloned human transferases to determine the chemical structure accepted as substrates (9).

Glucuronidation of drug molecules containing a wide range of acceptor groups has been reported including phenols (e.g., propofol, paracetamol, naloxone), alcohols (e.g., chloramphenicol, codeine, oxazepam), aliphatic amines (e.g., ciclopiroxalamine, lamotrigine, amitriptyline), acidic carbon atoms (e.g., feprazone, phenylbutazone, sulfinpyrazone), and carboxylic acids (e.g., naproxen, zomepirac, ketoprofen). This indicates the variability of acceptor groups that can be conjugated to glucuronic acid in humans (3).

UGT1*6 exhibited a limited substrate specificity for planar phenolic compounds, whereas UGT 1*02 was more promiscuous in acceptance of nonplanar phenols, anthraquinones, flavones, aliphatic alcohols, aromatic carboxylic acids, steroids, and many drugs of varied structure.

A cloned human bilirubin UGT (UGT 1*1) accepted a wide diversity of compounds such as phenols, anthraquinones, flavones, and steroids. Seven substrates were glucuronidated at rates comparable to or higher than bilirubin. Octyl gallate was glucuronidated at the highest rate among all substrates tested. The UGT1*1 isoform exhibited stereospecificity towards simple nonplanar phenols and estrogens. The increasing number of carbon atoms in the alkyl side chain of gallate analogues improved the specific activity from no glucuronide formed with gallic acid to 0.72 and 1.90 mmol/min/mg protein in propyl and octyl gallate, respectively (15). Seventeen steroids were tested and only four that had both 17 β - and 3 β -hydroxy substituents were glucuronidated (15).

Recently, UGT1*4 has been shown to catalyze the *N*-glucuronidation of drugs and xenobiotics (9). Subfamily two contains at least five UGTs catalyzing steroid or bile acid glucuronidation UGT-2B4 and UGT-2B7 (steroid/bile acid UGTs) also catalyzed the glucuronidation of some xenobiotics (9). Levels of UGT activity

were sufficient to allow determination of kinetic parameters for the enzyme reaction. Further, metabolism and toxicity of drugs could be studied by their addition to recombinant cell lines in culture, and extraction of the media allowed analysis of glucuronide formation. Suggested probe substrates for individual UGT isoforms are listed in Table 1.

Use of Antibodies to Validate Functional Specificity of Human UGTs in Human Liver Microsomes

Some problems in the use of expressed human drug-metabolizing enzymes in the analysis of drug metabolism and drug-drug interactions have been discussed in a recent commentary (8). The value of these *in vitro* systems is their relevance to human drug metabolism *in vivo*.

Recently, attempts have been made to assess the contribution of a specific UGT to the glucuronidation of an endobiotic or xenobiotic in a particular tissue by using inhibitory antibodies. Monospecific polyclonal antibodies raised against the N-terminal portion (14–150 residues) of human liver UGT2B4 protein expressed in *E. coli* were used to immunoinhibit and immunoprecipitate this transferase from human liver and kidney microsomes (16). These experiments demonstrated that UGT2B4 activity was responsible for more than 90% of the hydoexycholeate 6-*O*-glucuronidation activity in human liver microsomes, but did not contribute significantly toward the glucuronidation of estriol, 4-hydroxysterone, 1-naphthol, or hydoexycholeic acid (16).

Similarly, antibodies were raised against the N-terminal half of UGT1*6 expressed in *E. coli*, and immunoinhibition analysis of human liver microsomes demonstrated that this isoenzyme represented between 20 and 50% of the total microsomal 1-naphthol glucuronidation (17). UGT activities towards hydoexycholeic acid, 4-hydroxybiphenyl, 4-*t*-butylphenol, and bilirubin were not inhibited by these specific anti-UGT1*6 antibodies.

Table 1. Probe substrates for human UDP-glucuronosyltransferases.

UGT-1 Subfamily (chromosome 2q37)		UGT-2 Subfamily (chromosome 4q13)	
Isoenzyme	Substrate	Isoenzyme	Substrate
UGT1*1	Bilirubin	UGT2B4	Hydoexycholeic acid
UGT1*4	Imipramine		
UGT1*6	1-Naphthol	UGT2B8	Androstanediol
UGT1*02	Propofol		

Biologically Active and Potentially Toxic Glucuronides

Glucuronidation has been described as a safe detoxication process and glucuronides were never considered biologically active intermediates. However, in recent years the potential toxicity and biological activity of certain glucuronides have been well recognized.

There are several notable examples reported in which such metabolites have been found to be pharmacologically active. (–)-Morphine is glucuronidated in a stereoselective manner to (–)-morphine-3-glucuronide and (–)-morphine-6-glucuronide in the liver (18). Detailed pharmacologic characterization of the glucuronides has established that (–)-morphine-6-glucuronide is 650 times more potent as an analgesic than the parent drug, whereas morphine-3-glucuronide is a potent antagonist of morphine and has no analgesic activity (18). This discovery has led to (–)-morphine-6-glucuronide being commercially marketed.

The Role of Acyl Glucuronidation in Drug Immune Hypersensitivity

Acyl glucuronides are formed when conjugation with glucuronic acid occurs via a carboxyl group, resulting in an ester-type linkage. Ester-type glucuronides are much more unstable than ether-linked glucuronides and can easily undergo nucleophilic substitution. The chemical properties of acyl-linked glucuronides are extensively reviewed (19).

One important reaction that acyl glucuronides undergo is acyl migration, a process whereby the aglycone moves from the 1-hydroxyl group of the glucuronic acid sugar to the 2-, 3-, or 4-hydroxyl groups. This rearrangement of the glucuronide leads to β -glucuronidase-resistant isomers and is completely reversible with one exception: the C1-glucuronide does not appear to reform from the C2-isomer (19). The extent of acyl migration may only become detectable when the excretion of conjugates is impaired and their plasma concentrations are raised (19).

The rate of acyl migration differs from compound to compound and their stability is also highly variable (19). At physiological or slightly alkaline pH, acyl migration and hydrolysis of acyl glucuronides is extensive (19).

A number of acyl glucuronides have been shown to bind irreversibly to proteins *in vitro* and *in vivo* (20). Evidence exists

for two principal mechanisms of this reversible (covalent) binding; however, it is not known which of these is principally responsible (21).

Known Polymorphisms of Glucuronidation in Man

Polymorphic drug glucuronidation in man has not been extensively studied due to problems in the selection of suitable drugs for screening. The identification and measurement of metabolites, including the interaction between pathways of glucuronidation and sulfation, complicate interpretation of the data. The known polymorphisms are listed in Table 2.

Codeine glucuronidation was decreased in Han Chinese when compared to a Swedish population (22). Paracetamol glucuronidation in small Chinese and Caucasian groups has been compared but no interethnic differences were observed (23). In a more recent study of paracetamol glucuronidation, the distribution in a random population appeared to be unimodal although skewed (24). Glucuronidation was also clearly affected by gender, oral contraceptives, steroids, and smoking, further indicating the problems in determination of genetic polymorphism (24).

Liu et al. (25) reported that the glucuronide excretion of the hypolipidemic drug clofibrate in a healthy Caucasian population followed a normal distribution, whereas that of fenofibrate appeared to be distributed into the distinct normal groups. However, a follow-up familial study has shown lack of a genetic polymorphism in the glucuronidation of fenofibrate (26).

Another study, of a French population, examined variation of dextrorphan glucuronidation. Again, a normal distribution was observed (27). Analysis of the polymorphic variation of diflunisal phenol and acyl glucuronide showed a unimodal population distribution, especially when females using oral contraceptives were excluded (28).

Recently, oxazepam administered as a racemic mixture was shown to be preferentially excreted as the (S)-glucuronide and a low S/R glucuronide ratio was used to assess poor glucuronidation of oxazepam (29). A

group of 10% of the whole population was determined to be poor glucuronidators of (S)-oxazepam which suggested a genetic relationship to the UGT2B7 isoform. However, oxazepam may not be solely glucuronidated by UGT2B7 *in vivo* and this relationship requires additional investigation. Nonetheless, this is the most interesting example of a polymorphism of drug glucuronidation to date.

Menthol glucuronidation is considerably reduced in many patients with Crigler-Najjar syndrome, such that the menthol excretion test is often used as a confirmatory diagnosis of the disorder and as a study of the genetic inheritance of the disease (30,31). In a study of a family in which two CN children were born from the same marriage, only 5 of 16 members of the family showed a normal menthol glucuronide output. This menthol test demonstrated likely heterozygotes in the family members who were not revealed by serum bilirubin analyses (30). In other studies the test failed to discriminate between icteric patients and anicteric family members suspected to be heterozygotes. Indeed, jaundiced patients may show normal menthol glucuronide excretion (32). Bloomer et al. (33) also detected normal menthol conjugation in the parents of a Crigler-Najjar child. These accumulated data suggest that menthol glucuronidation is independently variable within the population.

Menthol is conjugated with glucuronic acid prior to excretion in bile and urine (31) and is therefore an interesting, relatively harmless test compound for study of polymorphism of drug glucuronidation in man. However, glucuronidation of menthol was only reduced to about 20% of controls in some of the Crigler-Najjar type 1 patients. This suggests that menthol may be a substrate for more than one UGT isoenzyme, which may complicate studies of menthol glucuronidation in the normal population.

The known defects of glucuronidation in humans are best illustrated within the group of a hereditary hyperbilirubinemia, (5) in which molecular genetic studies have revealed the association between genetic defects and loss of function.

Table 2. Polymorphisms of human UGT genes.

Gene	Defect	Substrate/Activity	Remarks
UGT1	Exons 1*1,2,3,4,5	Bilirubin reduced to 0–10%	Crigler-Najjar syndrome (1,2)
UGT1	5'-noncoding promoter region	Bilirubin reduced to 35%	Gilbert's disease patients
UGT1	Unknown	Menthol reduced to 20%	Linked with Crigler-Najjar
UGT2B7	Unknown	(S)-Oxazepam, valproate reduced	

Crigler-Najjar Syndrome

Crigler-Najjar syndrome (CN) is a familial form of severe unconjugated hyperbilirubinemia caused by a dysfunction in bilirubin glucuronidation. The molecular basis of these syndromes has recently been characterized by enzymological, immunochemical, and molecular genetic analysis. *In vitro* analysis of CN type 1 liver samples has revealed that as well as showing complete absence of bilirubin UGT activity, some of these patients also poorly glucuronidate phenols, 5-hydroxytryptamine, and the drugs ethinylestradiol and propofol (3).

Recently, as a result of the elucidation of the *UGT1* gene complex, several groups have determined some of genetic lesions that cause CN (5). The mutations that result in CN syndrome have been found in the exons 1,2,3,4, and 5 encoding the constant region of all *UGT1* proteins, which explains the decreased activity towards other aglycones as well as bilirubin (5). More than 20 genetic lesions have been demonstrated to be associated with the severe hyperbilirubinemia of CN (5).

Gilbert's Syndrome

Gilbert's syndrome (GS), a familial hyperbilirubinemia determined to be present in up to 5% of the population in 1980, is characterized by a mild unconjugated hyperbilirubinemia (34). Decreased formation of bilirubin diglucuronide and increased levels of bilirubin monoglucuronide were found in bile in parallel with decreased hepatic UGT activity (35). This disease provides an opportunity to study variation in drug glucuronidation due to the prevalence of the familial disorder within the population. There is no obvious indication of impaired drug oxidation, acetylation, or sulfation (36). Decreased clearance of several drugs, such as tolbutamide, rifamycin, josamycin, and paracetamol, has been observed (37), although decreased clearance was not apparently associated with a decreased rate of glucuronidation measured in overnight urine samples (36).

De Morais et al. (38) have reported that paracetamol glucuronide formation, measured in six GS patients by clearance from plasma within 2 hr, was 31% lower than in normal controls. The timing of measurements may be critical in determination of these significant differences.

Recent work has shown that the mildly affected members of families in which CN type 2 occurs are heterozygous for mutations in the *UGT* subfamily 1

(*UGT1* genes) may be classified as GS (39). However, the incidence of CN-2 in the population is very rare and the frequency of alleles causing CN-2 would not be sufficient to explain the population incidence of GS.

A study by Bosma et al. suggested a correlation between homozygosity for a 2bp insertion in the TATA box upstream of *UGT1*1* exon 1 and GS; no mutations were found in the coding sequence of the *UGT1*1* gene (40). We demonstrated that the primary genetic factor contributing to the variation in the serum and total bilirubin concentration in an eastern Scottish population is the sequence variation reported by Bosma et al. (40); a direct correlation is only revealed by a controlled study of a drug-free, alcohol-free nonsmoking population (41). We showed that the 7/7 genotype is associated with GS and occurs in 10 to 13% of the population (41).

Drugs, alcohol, and smoking induce human bilirubin UGT (41) and thereby interfere with the phenotype in the general population, creating latent GS patients.

It is now recognized that there are better xenobiotic substrates of *UGT1*1* (the major bilirubin-metabolizing form) than bilirubin itself, examples including octyl gallate and emodin (15), which obviously have the potential to cause jaundice by competitive inhibition of *UGT1*1*, especially in GS patients in whom hepatic activity is reduced to 35% of normal levels.

Xenobiotic Induction and Physiological Perturbation in Humans

Induction of glucuronidation reactions has also been reported in humans. (3). Indoles present in cruciferous vegetables (brussels sprouts and cabbage) appear to modestly enhance oxazepam and paracetamol glucuronidation (42). The anticonvulsant agents phenobarbitone, phenytoin, and carbamazepine either separately or in combination induce the glucuronidation of paracetamol (43) and possibly norcodeine (24). Carbamazepine also induces valproic acid glucuronidation (44). Coadministration of phenobarbitone and phenytoin induces chloramphenicol glucuronidation (45). Several oral contraceptive drugs increase the glucuronidation of paracetamol (24), clofibrilic acid (46), and temazepam (47).

Cimetidine has been shown to specifically increase the urinary excretion of naproxen acyl glucuronide, in contrast to other naproxen glucuronide metabolites (48).

Dexamethasone and phenobarbital cause up to a 2-fold induction of digitoxigenin monodigitoxoside UGT in human liver (49). Ethanol treatment of a GS patient led to a 3-fold decrease in serum bilirubin associated with a corresponding 2.5-fold increase in hepatic bilirubin UGT activity (50), and bilirubin UGT mRNA, encoded by the *UGT1*1* gene, was reported to be selectively induced in human livers from patients treated with phenytoin and phenobarbital (51). Indeed, widespread consumption of therapeutic and social drugs, e.g., ethanol, and exposure to environmental chemicals induce UGTs and may mask genetic variation, for example in GS patients (above).

Thyroid Hyperplasia

Plasma thyroxine (T₄) concentrations are monitored by the pituitary gland; if thyroxine levels decrease, the pituitary secretes thyroid-stimulating hormone (TSH) that increases the production of thyroxine by the thyroid follicular cells. TSH synthesis is under negative feedback regulation by thyroxine; hence, when plasma thyroxine concentrations return to normal TSH secretion stops. However, continuous depression of plasma thyroxine results in sustained increases of plasma TSH, leading to follicular cell proliferation, hyperplasia, and ultimately neoplasia (52). Plasma thyroxine levels may be decreased by either direct inhibition of its synthesis or by increased metabolism and excretion. A major route of thyroxine metabolism is conjugation with glucuronic acid in the liver, followed by biliary excretion.

Rats given four microsomal enzyme inducers had elevated UGT activity toward T₃ and T₄ of up to 60%, and the circulating T₃ and T₄ concentrations fell to 70 to 75% of control levels. Furthermore this reduction in hormone levels was not mediated by the thyroid (53). This effect results in continuous TSH synthesis and thyroid hyperplasia. This hyperplastic mechanism is one that is potentially operative in many species including humans. Glucuronidation of thyroxine is catalyzed by bilirubin UGT and other transferases in human liver (54). Therefore chemical induction of bilirubin UGT by alcohol and other drugs could increase risk of hypothyroidism and subsequent thyroid hyperplasia.

Xenobiotic Inhibition of Glucuronidation

In intact cell preparations (isolated cells, perfusions, *in vivo*) glucuronidation may

be inhibited by either a decrease of UDPGA availability or by inhibition decrease of UGT activity. No highly efficient, selective inhibitors of glucuronidation *in vivo* or in intact cells are available at present, although Mulder et al. (55) present a fairly recent review.

The studies of inhibition of UGTs are difficult and confused by the microsomal membrane location of transferases. Many compounds that have been considered inhibitors of glucuronidation cause membrane disruption or reduce cellular UDPGA concentrations (56).

Specific analysis is perhaps more easily achieved by measurement of endogenous compound glucuronidation such as the inhibition of bilirubin UGT activity. Gentamycin was shown to be a weak inhibitor of bilirubin UGT *in vitro*, but also inhibited salicylamide glucuronidation *in vivo* (57). Bilirubin has been shown to specifically inhibit 3 α -hydroxyestradiol glucuronide formation, but not 17 β -hydroxyestradiol glucuronide formation by human liver microsomes, indicating a stereospecific role of bilirubin UGT in formation of this endogenous glucuronide (15). Novobiocin, which caused unconjugated hyperbilirubinemia in animals and man, was shown to exert noncompetitive inhibition of rat microsomal UDP-glucuronosyltransferase in digitonin-activated preparations with either bilirubin or UDP-glucuronic acid. Human microsomal bilirubin UGT activity was also inhibited by novobiocin, whereas 1-naphthol UDP-glucuronosyltransferase was unaffected (58). Further studies with purified rat liver bilirubin UGT suggested that novobiocin competitively inhibited bilirubin binding to the enzyme, but the drug did not appear to be a substrate for the purified enzyme.

D-ring glucuronides of estriol, testosterone, and dihydrotestosterone possess pharmacological activity and mediate cholestasis (59). In contrast, A-ring conjugates of these steroids are inactive (60). Human bilirubin UGT is the major enzyme responsible for β -estradiol-3-glucuronide formation in human liver (15). Therefore, the bile ductular cholestasis described in CN patients might be due to the genetic defect in the human bilirubin UGT, which in turn results in excess formation of the cholestatic E₂-17 β -glucuronide rather than the noncholestatic E₂-3-glucuronide (61).

Androsterone glucuronide is the predominant C-19 steroid glucuronide in plasma (62). The marked rise in

testosterone during puberty was strongly correlated with increases of androsterone glucuronide and androstane-3 α , 17 β -diol glucuronide. Both steroid glucuronides are plasma biochemical markers of adrenal hyperandrogenism in hirsutism in women (63) and in virilizing congenital adrenal hyperplasia (64). Direct inhibition of UGTs by competitive xenobiotics could significantly affect the production of steroid glucuronides and, in turn, the physiological function responsive tissues such as ovary.

Tertiary amine drugs, chlorpromazine, amitriptyline, imipramine, promethazine, and cyproheptadine were potent inhibitors of the glucuronidation of testosterone, androsterone, and estriol. Structural features required for inhibition were a rigid tricyclic ring and either a dimethylaminopropyl or a methylpiperidine side chain (65).

Ketoprofen and other substrates of human UGT2B7 are competitive inhibitors of (*S*)-oxazepam glucuronidation in human liver microsomes, but not effective inhibitors of (*R*)-oxazepam glucuronidation, indicating a stereoselective, competitive inhibition of oxazepam glucuronidation, which may be catalyzed by two different UGTs (66).

Organization and Characterization of the Human Sulfotransferase Family

In man, five ST gene products have been identified and characterized and, as is the case for most mammalian STs, these can be classified into two distinct subfamilies (based on their amino acid sequence identities and substrate specificities): phenol ST (PST) and hydroxysteroid ST (HST) (Table 3) (67,68). PSTs, particularly M-PST and P-PST, sulfate numerous xenobiotics, thyroid hormones, and biogenic amines; the family also includes the estrogen ST (EST) that sulfates both endogenous and xenobiotic estrogens. A (to date) single but somewhat promiscuous human HST isoform exists, which sulfates many diverse steroids, bile salts, cholesterol, and

xenobiotic alcohols. It is likely, however, that other human STs exist, since additional STs are present in other mammalian species, for example, a member of the rat PST family (ST1C1) that shows remarkable specificity for aromatic hydroxylamines and hydroxamic acids (69). A new nomenclature system for the classification of all STs is currently being devised, which will be generally in line with those adopted for the cytochromes P450 and the UGTs.

cDNAs encoding all the known human STs have been isolated, sequenced, and expressed either in bacterial or mammalian cell-based heterologous expression systems. These are proving valuable in defining the boundaries of substrate specificity of the various forms. In general, the kinetic and catalytic properties of these recombinant, expressed STs are as expected. There appears to be some microheterogeneity at the amino acid sequence level with certain STs, in particular with the P-PST.

cDNAs encoding this isoform have been isolated in a number of laboratories, and at least three allelic variants differing by up to five amino acids seem to be present within the population. The effects of these variants on the substrate specificity and kinetic parameters of P-PST remain to be determined. A P-loop motif (GXXGXXK) located towards the C-terminus is conserved in all sulfotransferases, and this has been demonstrated to be involved in binding the cosubstrate, 3'-phosphoadenosine 5'-phosphosulfate (PAPS) (70). Site-directed mutagenesis experiments are beginning to identify amino acids important for catalysis (71).

Each ST appears to be transcribed from a single gene, although there is some evidence for alternate splicing from 5' noncoding "exons" that may play a role in tissue-specific expression. STs are expressed in many tissues, with marked selectivity for the different forms. P-PST appears almost ubiquitous and the liver is a major site for P-PST, HST, and EST expression, although M-PST is a minor form in this tissue. This is in contrast to the small intestine, where M-PST appears to be the major form. HST plays a

Table 3. The human sulfotransferase family.

ST isoform	Subfamily	Chromosome	Prototypical substrates	References
P-PST (ST1A3)	PST	16p	4-Nitrophenol, minoxidil	Dooley et al. (92)
ST1A2	PST	16p	4-Nitrophenol, minoxidil	Her et al. (95)
M-PST	PST	16p	Dopamine, 5-hydroxytryptamine	Dooley et al. (92)
EST	PST	4q	β -estradiol, 17 α -ethinylestradiol	Her et al. (93)
HST	HST	19q	Dehydroepiandrosterone, pregnenolone	Ottersen et al. (94)

fundamental biological role during development, as it is responsible for the production of dehydroepiandrosterone sulfate by the fetal adrenal gland during the second half of pregnancy (72), which in turn is the major substrate for placental estrogen biosynthesis. Both M- and P-PST are expressed in the brain, although the function remains unclear. Platelets, which contain P-PST and M-PST but not EST or HST proteins, are one of the most widely studied tissues, although their relevance to other tissues has remained a matter of some debate.

The availability of good quality, isoform-specific antibodies is essential for the thorough characterization and analysis of any enzyme family, and this has proven particularly difficult for the STs. Numerous antibodies have been raised against, in particular, rat and human STs, but the high degree of sequence identity among the three known human PSTs (P-PST, ST1A2, and M-PST) means that antibodies distinguishing between them have so far eluded the field. It was demonstrated that synthetic peptides could be used to induce antibodies against human HST and members of the PST family (73), and advancement of this work has now yielded the first antibodies specific for M-PST and for P-PST (Rubin et al., unpublished data).

Biologically Active and Toxic Sulfates

It has been known (but perhaps not widely) for many years that sulfation does not always result in a reduction in biological activity, from Millers' pioneering work on the role of sulfation in the bioactivation of chemical procarcinogens (7). For the anti-hypertensive and hypertrichotic drug minoxidil, bioactivation by sulfation is beneficial, since the sulfate ester is the pharmacologically active species (74). Elegant experiments demonstrated that the ST responsible for this reaction is expressed in the hair follicles, providing *in situ* activation of topically applied minoxidil (75), which has found clinical application in the treatment of baldness.

A classical pathway of bioactivation of aromatic amines such as 2-aminofluorene involves *N*-hydroxylation by cytochrome P450 (forming an aromatic hydroxylamine) followed by reaction with PST to form a highly unstable *N*-*O*-sulfate that rapidly and spontaneously decomposes, resulting in the formation of a highly reactive aryl nitrenium ion that is able to adduct to DNA and proteins.

The requirement for sulfation in the bioactivation of promutagens can be demonstrated *in vitro* using the Ames test (76). There is also a significant amount of good (albeit circumstantial) evidence for the key role of sulfation in the bioactivation of certain chemical procarcinogens *in vivo*. The brachymorphic mouse, which has a dramatically reduced capacity for PAPS synthesis, is remarkably resistant to chemical-induced cancer when fed compounds (such as aromatic amines) that require sulfation for activation to mutagens (7). Male rats are much more susceptible to hepatocarcinogenesis following exposure to aromatic amines than female rats, and male rats have up to 10-fold higher levels of expression of the PST(s), which sulfate aromatic hydroxylamines; and the carcinogenicity of aromatic amines is much reduced when coadministered with potent inhibitors of PSTs (7). Human liver PST(s) are primarily responsible for the sulfation of aromatic hydroxylamines and hydroxamic acids (77), whereas benzylic alcohols of polycyclic aromatic hydrocarbons (which are also conditional on sulfation for bioactivation) are preferentially sulfated by HST (78).

The upsurge of interest in sulfation and the STs involved in these bioactivation pathways is principally a result of two factors. First, the demonstration that the hydroxylated metabolites of heterocyclic amines formed during the cooking of meat and fish, which are widely believed to play an important role in colon cancer, are substrates for PST(s) (79,80). Second, there appears to be polymorphic expression of P-PST within the human population. An individual's genetic complement of STs may therefore be an important risk factor in susceptibility to cancer of the colon and possibly other tissues. Testing this hypothesis is therefore an important health priority.

Known Polymorphisms of Sulfation in Man

Platelet P-PST and M-PST activity varies widely within the population (81). Much of the work on sulfation pharmacogenetics has been done using the platelets because they are a readily accessible tissue and because the level of P-PST in platelets appears to correlate with that in other tissues from the same individual, in particular the cerebral cortex, small intestinal mucosa and liver (81). Platelets, of course, are unlikely to play a role in drug metabolism; the simple measurement of enzyme activity

may not provide the whole picture, since there are several closely related PSTs known to exist in humans with undoubtedly overlapping substrate specificity. The discovery of isoenzyme-selective substrates is therefore critical although potentially very difficult when P-PST (ST1A3) and ST1A2 share 96% amino acid identity (67). The difficulties attached to producing molecular probes to distinguish the various forms at the DNA, mRNA, or protein level are also considerable.

Classical enzyme activity measurements in platelets from a large number of individuals and within families have suggested a significant heritability for both P- and M-PST, and polymorphic expression of the P-PST form in this tissue (81). The correlation between P-PST enzyme activity and enzyme protein levels in platelets has also been shown (81). Similarly, statistically significant correlations between the extent of paracetamol (acetaminophen) sulfation *in vivo* and the levels of platelet M-PST and P-PST enzyme activity (paracetamol is a substrate for both enzymes) have been demonstrated (82), although this drug is also subject to other metabolic routes.

There is also population variability in the expression of human HST, with an apparent bimodal distribution of enzyme activity in a series of 94 liver samples, although the variation was only 4.6-fold (83). Recent sequence analysis of the gene coding for HST indicated the presence of restriction fragment length polymorphisms and nucleotide mutations within the population, and such analyses will facilitate the study of molecular mechanisms of ST regulation in man (84).

Regulation of Sulfotransferases by Xenobiotics

Sulfotransferases are considered to be refractory to induction by "classical" xenobiotic inducers. Recent analysis of the effect of peroxisome proliferators on rat liver ST expression demonstrated a suppression of the major rat PSTs ASTIV (or ST1A1) and ST1C1 (85), and the suppression of rat liver HST expression after treatment with 3-methylcholanthrene has also been shown (86).

Xenobiotic Inhibition of Sulfation and Human Risk

Inhibition in Intact Cells and *in Vivo*

Sulfation can be inhibited either by direct inhibition of the enzymes or by interfering

with the biosynthesis of the cosubstrate PAPS through, for example, reduction in inorganic sulfate availability. 2,6-Dichloro-4-nitrophenol (DCNP) and pentachlorophenol (PCP) are potent inhibitors of PST activity (in humans, specifically of the P-PST isoform[s]). Inhibition of sulfation *in vivo*, in perfused organs and intact cells, and *in vitro* can be achieved with these chemicals and with many other compounds (87).

Inhibition of ST Activity *in Vitro* by Drugs and Dietary Compounds

The important role of sulfation in modulating the biological activity of key endobiotics and the fact that all human STs number both xenobiotics and endobiotics among their substrates mean that interference with this function by inhibition may have important consequences for the body's physiology and biochemistry.

The compounds DCNP and PCP, particularly potent and selective inhibitors of human P-PST *in vitro*, were used in the pre-recombinant DNA technology era as tools for classification of the different human STs. Studies on the inhibition of human liver steroid sulfation by a wide

range of drugs demonstrated that a number of commonly used pharmaceuticals were potent inhibitors of the sulfation of DHEA by HST and the sulfation of estrone by EST (88). Clomiphene, danazol, spironolactone, cyproterone, and chlorpromazine were all able to inhibit HST activity with an IC₅₀ value of less than 10 μ M, and EST activity was inhibited by cyclizine, ibuprofen, chlorpheniramine, dimenhydrinate, and tamoxifen, again with IC₅₀ values < 10 μ M. These observations have implications for adverse drug reactions; but their effects *in vivo*, either in humans or animals, remains to be determined. Another obvious source of xenobiotic inhibitors is the diet, and a number of natural and synthetic dietary chemicals were shown to potentially inhibit the sulfation of 17 α -ethinylestradiol and dopamine (89). These included vanillin (a naturally occurring flavoring), octyl gallate (an antioxidant), and tartrazine (a synthetic colorant). A number of flavonoids, such as quercetin, are also potent inhibitors of ST activity (90); and red wine, which contains very high levels of polyphenolic compounds, is a very potent and selective inhibitor of human

P-PST with a 2000-fold dilution resulting in 50% inhibition of this enzyme activity (91). The importance of these dietary inhibitors may lie in their ability to inhibit the bioactivation of dietary procarcinogens by STs (particularly P-PST) and thus act as natural chemoprotectants.

Concluding Remarks

The "undergraduate textbook" concept of glucuronidation and sulfation as purely detoxication mechanisms is clearly outdated. As we understand more about these complex and fascinating enzyme systems, it becomes increasingly clear that there is a fine balance between their beneficial and harmful properties. The combination of an individual's genetically determined complement of these, and other, xenobiotic metabolizing enzymes and the environmental pressures upon them are obviously major factors in determining which way the balance tips. Not until we have learned much more of the structure/function relationships, regulation and pharmacogenetics of the UGTs and STs, however, will we be able to fully exploit their potential as risk assessment tools.

REFERENCES

- Dutton GJ, Burchell B. Newer aspects of glucuronidation. *Prog Drug Metab* 2:1-70 (1977).
- Weinshilboum R, Otterness D. Sulfotransferase enzymes. In: *Conjugation-Deconjugation Reactions in Drug Metabolism and Toxicity*. Handbook Experimental Pharmacology (Kauffman FC, ed). Berlin:Springer-Verlag, 1994:45-78.
- Clarke DJ, Burchell B. The uridine diphosphate glucuronosyltransferase multigene family: function and regulation. *Handbook Exper Pharmacol* 112:3-43 (1994).
- Tephly TR, Burchell B. The UDP-glucuronosyltransferases: a family of detoxifying enzymes. *Trends Pharmacol Sci* 11:276-279 (1990).
- Burchell B, Coughtrie MWH, Jansen PLM. Function and regulation of UDP-glucuronosyltransferase genes in health and liver-disease—Report of the 7th International Workshop on Glucuronidation, September 1993, Pitlochry, Scotland. *Hepatology* 20:1622-1630 (1994).
- Hobkirk R. Steroid sulfation—current concepts. *Trends Endocrinol Metab* 4:69-74 (1993).
- Miller JA. Sulfonation in chemical carcinogenesis—history and present status. *Chem Biol Interact* 92:329-341 (1994).
- Rommel RP, Burchell B. Validation and use of cloned, expressed human drug metabolising enzymes in heterologous cells for analysis of drug metabolism and drug-drug interactions. *Biochem Pharmacol* 46:559-566 (1993).
- Burchell B, Brierley CH, Rance D. Specificity of human UDP-glucuronosyltransferases and xenobiotic glucuronidation. *Life Sci* 57:1819-1831 (1995).
- Brierley CH, Burchell B. Human UDP-glucuronosyltransferases: chemical defence, jaundice and gene therapy. *Bioessays* 15:749-754 (1993).
- Owens IS, Ritter JK. The novel bilirubin/phenol UDP-glucuronosyltransferase *UGT1* gene locus: implications for multiple nonhemolytic familial hyperbilirubinemia phenotypes. *Pharmacogenetics* 2:93-108 (1992).
- Wooster R, Sutherland L, Ebner T, Clarke DJ, Da Cruz e Silva O, Burchell B. Cloning and stable expression of a new member of the human liver phenol/bilirubin UDP-glucuronosyltransferase cDNA family. *Biochem J* 278:465-469 (1991).
- Moghrabi N, Sutherland L, Wooster R, Povey S, Boxer M, Burchell B. Chromosomal assignment of human phenol and bilirubin UDP-glucuronosyltransferase genes (*UGT1A* subfamily). *Ann Hum Genet* 56:83-93 (1992).
- Monaghan G, Clarke DJ, Povey S, Gee See C, Boxer M, Burchell B. Isolation of a human YAC contig encompassing a cluster of *UGT2* genes and its regional localization to chromosome 4q13. *Genomics* 23:496-499 (1994).
- Senafi SB, Clarke DJ, Burchell B. Investigation of the substrate-specificity of a cloned expressed human bilirubin UDP-glucuronosyltransferase—UDP-sugar specificity and involvement in steroid and xenobiotic glucuronidation. *Biochem J* 303:233-240 (1994).
- Pillot T, Ouazzine M, Fournel-Gigleux S, Lafaurie C, Radominska A, Burchell B, Siest G, Magdalou J. Glucuronidation of hyodeoxychoic acid in human liver—evidence for a selective role for UDP-glucuronosyltransferase-2B4. *J Biol Chem* 268:25636-25642 (1993).
- Ouazzine M, Pillot T, Fournel-gigleux S, Magdalou J, Burchell B, Siest G. Expression and role of the human liver UDP-glucuronosyltransferase *UGT1*6* analyzed by specific antibodies raised against a hybrid protein produced in *Escherichia coli*. *Arch Biochem Biophys* 310:196-204 (1994).
- Paul D, Standifer KM, Inturrisi CE, Pasternak GW. Pharmacological characterization of morphine-6- β -glucuronide, a very potent morphine metabolite. *Pharmacol Exp Therap* 251:477-483 (1989).

19. Sphann-Langguth H, Benet LZ. Acyl glucuronides revisited: Is the glucuronidation process a toxification as well as detoxification mechanism? *Drug Metab Rev* 24:5–48 (1992).
20. Boelsterli UA. Specific targets of covalent drug-protein interactions in hepatocytes and their toxicological significance in drug-induced liver injury. *Drug Metab Rev* 25:395–451 (1993).
21. Ding A, Ojingwa JC, McDonagh AF, Burlingame AL, Benet LZ. Evidence for covalent binding of acyl glucuronides to serum-albumin via an imine mechanism as revealed by tandem mass-spectrometry. *Proc Nat Acad Sci USA* 90:3797–3801 (1993).
22. Yue QY, Svensson JO, Alm C, Sioqvist F, Sawe J. Interindividual and interethnic differences in the demethylation and glucuronidation of codeine. *Br J Clin Pharmacol* 28:629–637 (1989).
23. Osborne NJ, Tonkin AL, Miners JO. Interethnic differences in drug glucuronidation: a comparison of paracetamol metabolism in Caucasians and Chinese. *Br J Clin Pharmacol* 32:765–767 (1991).
24. Bock KW, Schrenk D, Forster A, Griesse E, Morike K, Brockmeier D, Eichelbaum M. The influence of environmental and genetic factors on CYP2D6, CYP1A2 and UDP-glucuronosyltransferases in man using sparteine, caffeine, and paracetamol as probes. *Pharmacogenetics* 4:209–218 (1994).
25. Liu HF, Vincentviry M, Galteau MM, Gueguen R, Magdalou J, Nicolas A, Leroy P, Siest G. Urinary glucuronide excretion of fenofibric and clofibric acid glucuronides in man—is it polymorphic? *Eur J Clin Pharmacol* 41:153–159 (1991).
26. Vincent-Viry M, Cossy C, Galteau MM., Gueguen R, Magdalou J, Nicolas A, Leroy P, Siest G. Lack of a genetic polymorphism in the glucuronidation of fenofibric acid. *Pharmacogenetics* 5:50–52 (1995).
27. Duche JC, Querol-Ferrer V, Barre J, Mesangeau M, Tillement JP. Dextromethorphan O-demethylation and dextrorphan glucuronidation in a French population. *Int J Clin Pharmacol Ther Toxicol* 31: 392–398 (1993).
28. Herman RJ, Loewen GR, Antosh DM, Taillon MR, Hussein S, Verbeeck RK. Analysis of polymorphic variation in drug metabolism. III: Glucuronidation and sulfation of diflunisal in man. *Clin Invest Med* 17:297–307 (1994).
29. Patel M, Tang BK, Grant DM, Kalow W. Interindividual variability in the glucuronidation of (S) oxazepam contrasted with that of (R) oxazepam. *Pharmacogenetics* 5:287–297 (1995).
30. Arias IM. Chronic unconjugated hyperbilirubinemia without overt signs of hemolysis in adolescents and adults. *J Clin Invest* 41:2233–2245 (1962).
31. Szabo L, Ebrey P. Studies on the inheritance of Crigler-Najjar syndrome by the menthol test. *Acta Paediatr Scand* 4:153–158 (1963).
32. Arias IM, Gartner LM, Cohen M, Ezzer JB, Levi AJ. Chronic nonhemolytic unconjugated hyperbilirubinemia with glucuronyltransferase deficiency: clinical, biochemical, pharmacologic and genetic evidence for heterogeneity. *Am J Med* 47:395–409 (1969).
33. Bloomer JR, Berk PD, Howe RB, Berlin NI. Bilirubin metabolism in congenital nonhemolytic jaundice. *Pediatr Res* 5:256–264 (1971).
34. Fevery J. Pathogenesis of Gilbert's Syndrome. *Eur J Clin Invest* 11:417–418 (1981).
35. Fevery J, Blanckaert N, Heirwegh KP, Preaux AM, Berthelot P. Unconjugated bilirubin and an increased proportion of bilirubin monoconjugates in the bile of patients with Gilbert's syndrome and Crigler-Najjar disease. *J Clin Invest* 60:970–979 (1977).
36. Ullrich D, Sieg A, Blume R, Bock KW, Schrotter W, Bircher J. Normal pathways for glucuronidation, sulfation and oxidation of paracetamol in Gilbert's syndrome. *Eur J Clin Invest* 17:237–240 (1987).
37. Macklon AF, Savage RL, Rawlins MD. Gilbert's syndrome and drug metabolism. *Clin Pharmacokinet* 4:223–232 (1979).
38. De Morais SMF, Wells PG. Enhanced acetaminophen toxicity in rats with bilirubin glucuronyltransferase deficiency. *Hepatology* 10:163–167 (1989).
39. Koiwai O, Nishizawa M, Hasada K, Aono S, Adachi Y, Mamiya N, Sato H. Gilbert's syndrome is caused by heterozygous missense mutation in the gene for bilirubin UDP-glucuronosyltransferase. *Hum Mol Genet* 4:1183–1186 (1995).
40. Bosma PJ, Roy Chowdhury J, Bakker C, Gantla S, De Boer A, Oostra BA, Lindhout D, Tytgat GN J, Jansen PLM, Oude Elferink RPJ et al. The genetic basis of the reduced expression of bilirubin UDP-glucuronosyltransferase 1 in Gilbert's syndrome. *N Engl J Med* 333:1171–1218 (1995).
41. Monaghan G, Ryan, MF, Seddon R, Hume R, Burchell B. Genetic variation in bilirubin UDP-glucuronosyltransferase gene promoter and Gilbert's syndrome. *Lancet* 347:578–581 (1996).
42. Pantuck EJ, Pantuck CB, Anderson KE, Wattenberg LW, Conney AH, Kappas A. Effect of brussels-sprouts and cabbage on drug conjugation. *Clin Pharmacol Ther* 35:161–169 (1984).
43. Bock KW, Wiltfang J, Blume R, Ullrich D, Bircher J. Paracetamol as a test drug to determine glucuronide formation in man. Effects of inducers and of smoking. *Eur J Clin Pharmacol* 31:677–683 (1987).
44. Panesar SK, Orr JM, Farrell K, Burton RW, Kassahun K, Abbott FS. The effect of carbamazepine on valproic acid disposition in adult volunteers. *Br J Clin Pharmacol* 27:323–328 (1989).
45. Bloxham RA, Durbin GM, Johnson T, Winterborn MH. Chloramphenicol and phenobarbitone—a drug interaction. *Arch Dis Child* 54:76–77 (1979).
46. Miners JO, Robson RA, Birkett DJ. Gender and oral contraceptive steroids as determinants of drug glucuronidation: effects on clofibric acid elimination. *Br J Clin Pharmacol* 240:243 (1984).
47. Stoehr GP, Kroboth PD, Juhl RP, Wender DB, Phillips JP, Smith, RB. Effect of oral contraceptives on triazolam, temazepam, alprazolam and lorazepam kinetics. *Clin Pharmacol Ther* 36:683–690 (1984).
48. Vree TB, Van der Biggelaar-Martea M, Verwey Van Wissen CPWGM, Vree ML, Guelen PJM. The pharmacokinetics of naproxen, its metabolite O-desmethylnaproxen, and their acyl glucuronides in humans—effect of cimetidine. *Br J Clin Pharmacol* 35:467–472 (1993).
49. Schuetz EG, Hazelton GA, Hall J, Watkins PB, Klaassen CD, Guzelian PS. Induction of digitoxigenin monodigitoxoside UDP-glucuronosyltransferase activity by glucocorticoids and other inducers of cytochrome-P-450 in primary monolayer-cultures of adult rat hepatocytes and in human liver. *J Biol Chem* 261:8270–8275 (1986).
50. Ideo G, De Franchis R, Del Ninno E, Dioguardi N. Ethanol increases liver uridine-diphosphate-glucuronosyltransferase. *Experientia* 27:24–25 (1971).
51. Sutherland L, Ebner T, Burchell B. The expression of UDP-glucuronosyltransferase of the UGT1 family in human liver and kidney and in response to drugs. *Biochem Pharmacol* 45:295–301 (1993).
52. Saito K, Kameko H, Sato K, Yashitoke A, Yamada H. Hepatic UDP-glucuronosyltransferase activity towards thyroid hormones in rats: induction and effects on serum thyroid hormone levels following treatment with various enzyme inducers. *Toxicol Appl Pharmacol* 111:99–106 (1991).
53. Barter RA, Klaassen CD. Reduction of thyroid hormone levels and alteration of thyroid function by four representative UDP-glucuronosyltransferase inducers in rats. *Toxicol Appl Pharmacol* 128:9–17 (1994).
54. Visser TJ, Kaptein E, Gijzel AL, de Herder WW, Ebner T, Burchell B. Glucuronidation of thyroid hormone by human bilirubin and phenol UDP-glucuronosyltransferase isoenzymes. *FEBS Lett* 324:358–360 (1993).
55. Mulder GJ, Coughtrie MWH, Burchell B. Glucuronidation. In: *Conjugation Reactions in Drug Metabolism* (Mulder GJ, ed). London: Taylor and Francis, 1990:51–55.
56. Dutton GJ. *Glucuronidation of Drugs and Other Compounds*. Boca Raton, FL: CRC Press, 1980.
57. Malaka-Zafrii K, Tsiouris I, Cassimos C. The effect of gentamicin on liver glucuronosyltransferase. *J Pediatr* 82:118–120 (1973).

58. Burchell B, Coughtrie MWH, Jackson MR, Shepherd SRP, Harding D. Genetic deficiency of bilirubin glucuronidation in rats and humans. *Mol Aspects Med* 9:429–455 (1987).
59. Meyers M, Slikker W, Vore M. Steroid D-ring glucuronides: characterisation of a new class of cholestatic agents in the rat. *J Pharmacol Exp Ther* 218:63–73 (1981).
60. Slikker W, Vore M, Bailey JR, Meyers M, Montgomery C. Hepatotoxic effects of estradiol-17- β -D-glucuronide in the rat and monkey. *J Pharmacol Exp Ther* 225:138–143 (1983).
61. Vore M, Slikker W. Steroid D-ring glucuronides: a new class of cholestatic agents. *Trends Pharmacol Sci* 6:256–259 (1985).
62. Thompson DL, Horton N, Rittmaster RS. Androsterone glucuronide is a marker of adrenal hyperandrogenism in hirsute women. *Clin Endocrinol* 32:283–292 (1990).
63. Salman K, Spielvogel RL, Shulman LH, Miller JL, Vanderlinde RE, Rose LI. Serum androstenediol glucuronide in women with facial hirsutism. *J Am Acad Dermatol* 26:411–414 (1992).
64. Pang S, Macgillivray M, Wang M, Jeffries S, Clark A, Rosenthal I, Weigensberg M, Riddick L. 3 α -Androstenediol glucuronide in virilizing congenital adrenal hyperplasia: a useful serum metabolic marker of integrated adrenal androgen secretion. *J Clin Endocrinol Metab* 73:166–174 (1991).
65. Sharp S, Mak LY, Smith DJ, Coughtrie MWH. Inhibition of human and rabbit liver steroid and xenobiotic UDP-glucuronosyltransferases by tertiary amine drugs—implications for adverse drug reactions. *Xenobiotica* 22:13–25 (1992).
66. Patel M, Tang BK, Kalow W. (S) Oxazepam glucuronidation is inhibited by ketoprofen and other substrates of UGT 2B7. *Pharmacogenetics* 5:43–29 (1995).
67. Yamazoe Y, Nagata K, Ozawa S, Kato R. Structural similarity and diversity of sulfotransferases. *Chem Biol Interact* 92:107–117 (1994).
68. Coughtrie MWH. Sulfation catalysed by the human cytosolic sulfotransferases—chemical defence or molecular terrorism? *Hum Exp Toxicol* 15:547–555 (1996).
69. Nagata K, Ozawa S, Miyata M, Shimada M, Gong D-W, Yamazoe Y, Kato R. Isolation and expression of a cDNA encoding a male-specific rat sulfotransferase that catalyzes activation of *N*-hydroxy-2-acetylaminofluorene. *J Biol Chem* 268:24720–24725 (1993).
70. Chiba H, Komatsu K, Lee YC, Tomizuka T, Strott CA. The 3'-terminal exon of the family of steroid and phenol sulfotransferase genes is spliced at the N-terminal glycine of the universally conserved GXXGXXK motif that forms the sulfonate donor binding site. *Proc Natl Acad Sci USA* 92:8176–8179 (1995).
71. Driscoll WJ, Komatsu K, Strott CA. Proposed active-site domain in estrogen sulfotransferase as determined by mutational analysis. *Proc Natl Acad Sci USA* 92:12328–12332 (1995).
72. Barker EV, Hume R, Hallas A, Coughtrie MWH. Dehydroepiandrosterone sulfotransferase in the developing human fetus—quantitative biochemical and immunological characterization of the hepatic, renal, and adrenal enzymes. *Endocrinology* 134:982–989 (1994).
73. Sharp S, Coughtrie MWH, Forbes KJ, Hume R. Preparation and characterization of anti-peptide antibodies directed against human phenol and hydroxysteroid sulfotransferases. *J Pharmacol Toxicol Method* 34:89–95 (1995).
74. Meisneri KD, Cipkus LA, Taylor CJ. Mechanism of action of minoxidil sulfate-induced vasodilation: a role for increased K⁺ permeability. *J Pharmacol Exp Ther* 245:751–760 (1988).
75. Baker CA, Uno H, Johnson GA. Minoxidil sulfation in the hair follicle. *Skin Pharmacol* 7:335–339 (1994).
76. Glatt H, Pauly K, Czich A, Falany JL, Falany CN. Activation of benzylic alcohols to mutagens by rat and human sulfotransferases expressed in *Escherichia coli*. *Eur J Pharmacol Environ Toxicol* 293:173–181 (1995).
77. Gilissen RAHJ, Bamforth KJ, Stavenuiter JFC, Coughtrie, MWH, Meerman JHN. Sulfation of aromatic hydroxamic acids and hydroxylamines by multiple forms of human liver sulfotransferases. *Carcinogenesis* 15:39–45 (1994).
78. Glatt H, Pauly K, Frank H, Seidel A, Oesch F, Harvey RG, Werleschneider G. Substance-dependent sex differences in the activation of benzylic alcohols to mutagens by hepatic sulfotransferases of the rat. *Carcinogenesis* 15:2605–2611 (1994).
79. Chou HC, Lang NP, Kadlubar FF. Metabolic activation of *N*-hydroxy arylamines and *N*-hydroxy heterocyclic amines by human sulfotransferase(s). *Cancer Res* 55:525–529 (1995).
80. Ozawa S, Chou HC, Kadlubar FF, Yamazoe Y, Kato R. Activation of 2-hydroxyamino-1-methyl-6-phenylimidazo[4,5-*b*]pyridine by cDNA-expressed human and rat arylsulfotransferases. *Jpn J Cancer Res* 85:1220–1228 (1995).
81. Weinshilboum RM. Sulfotransferase pharmacogenetics. *Pharmacol Ther* 45:93–107 (1990).
82. Bonham-Carter SM, Rein G, Glover V, Sandler M, Caldwell J. Human platelet phenol sulfotransferase *M* and *P*: substrate specificities and correlation with *in vivo* sulfoconjugation of paracetamol and salicylamide. *Br J Clin Pharmacol* 15:323–330 (1983).
83. Aksoy IA, Sochorova V, Weinshilboum RM. Human liver dehydroepiandrosterone sulfotransferase—nature and extent of individual variation. *Clin Pharmacol Ther* 54:498–506 (1993).
84. Wood TC, Otterness DM, Weinshilboum RM. Human dehydroepiandrosterone sulfotransferase pharmacogenetics: restriction fragment length polymorphisms and gene sequence polymorphisms. [Abstract] *ISSX Proceedings* 8:111 (1995).
85. Witzmann F, Coughtrie MWH, Fultz C, Lipscomb J. Effect of structurally diverse peroxisome proliferators on rat hepatic sulfotransferase. *Chem Biol Interact* 99:73–84 (1996).
86. Rungemorris M, Wilusz J. Suppression of hydroxysteroid sulfotransferase- α gene-expression by 3-methylcholanthrene. *Toxicol Appl Pharmacol* 125:133–141 (1994).
87. Mulder GJ, Jakoby WB. Sulfation. In: *Conjugation Reactions in Drug Metabolism* (Mulder GJ, ed). London:Taylor and Francis, 1990:107–161.
88. Bamforth KJ, Dalglish K, Coughtrie MWH. Inhibition of human liver steroid sulfotransferase activities by drugs—a novel mechanism of drug toxicity. *Eur J Pharmacol* 228:15–21 (1992).
89. Bamforth KJ, Jones AL, Roberts RC, Coughtrie MWH. common food additives are potent inhibitors of human liver 17- α -ethinylestradiol and dopamine sulfotransferases. *Biochem Pharmacol* 46:1713–1720 (1993).
90. Walle T, Eaton EA, Walle UK. Quercetin, a potent and specific inhibitor of the human *P*-form phenolsulfotransferase. *Biochem Pharmacol* 50:731–734 (1995).
91. Jones AL, Roberts RC, Colvin DW, Rubin GL, Coughtrie MWH. Reduced platelet phenolsulfotransferase activity towards dopamine and 5-hydroxytryptamine in migraine. *Eur J Clin Pharmacol* 49:109–114 (1995).
92. Dooley TP, Mitchison HM, Munroe PB, Probst P, Neal M, Siciliano MJ, Deng Z, Doggett NA, Callen DF, Gardiner RM et al. Mapping of two phenol sulfotransferase genes *STP* and *STM* to 16p: candidate genes for Batten disease. *Biochem Biophys Res Commun* 205:482–489 (1994).
93. Her C, Aksoy IA, Kimura S, Brandriff BF, Wasmuth JJ, Weinshilboum RM. Human estrogen sulfotransferase gene (*STE*): cloning, structure, and chromosomal localization. *Genomics* 29:16–23 (1995).
94. Otterness DM, Mohrenweiser HW, Brandriff BF, Weinshilboum RM. Dehydroepiandrosterone sulfotransferase gene (*STD*): localization to human chromosome band 19q13.3. *Cytogenet Cell Genet* 70:45–47 (1995).
95. Her CT, Raftogianis R, Weinshilboum R. Human sulfotransferase pharmacogenetics—*STP2* gene, structural characterization and chromosomal localization. *Clin Pharmacol Ther* 59:216(abs)(1996).